

Morphology of exophytic ovipositors in dragonflies (Odonata: Gomphidae, Corduliidae, Libellulidae), with particular reference to ovipositor muscles and sensilla

Natalia A. Matushkina*

Department of Zoology, Biological Faculty, Kyiv National University, vul. Volodymirs'ka, 64, Kyiv, UA-01033, Ukraine

(Received 30 May 2011; final version received 09 August 2011)

A comparative study of female external genitalia was carried out in representatives of three dragonfly families that lay eggs exophytically, with special emphasis placed on skeletal musculature and sensilla. Female external genitalia are characteristically represented by the vulvar lamina and rudiments on the 9th sternum. In a gomphid, *Gomphus vulgatissimus*, and a corduliid, *Cordulia aenea*, the vulvar laminae bear numerous styloconic sensilla and sparse campaniform sensilla. In addition, the rudiments of *C. aenea* are richly furnished with basiconic sensilla, each with an apical pore. In corduliids and libellulids the ovipositor musculature is formed by two antagonistic muscles, contractions of which cause up- and downward movements of the middle part of the 9th sternum, where rudiments are usually located. Characteristically, gomphid females lack both the ovipositor-related muscles and rudiments. Based on the present results, the rudiments may be reasonably homologized with the gonapophyses of the 9th segment of the plesiomorphic well-developed ovipositor. The proposed functional interpretations of the ovipositor derivatives in Odonata with exophytic oviposition are discussed in light of their egg laying behaviour.

Keywords: female genitalia; musculature; vulvar lamina; gonapophysis; exophytic oviposition; homology; heterochrony; Odonata; Insecta

Introduction

“... The early evolution of an ensemble of egg-related characters ... enabled expansion into previously unavailable niches, and provided the Insecta with the potential for rapid and extensive phyletic divergence” (Zeh et al., 1989, p. 148). This “insect egg” hypothesis is undoubtedly supported by dragonfly oviposition. Dragonflies exhibit various patterns of egg laying behaviour. Two of these, endophytic and exophytic oviposition, represent the most common strategies of recent Odonata and illustrate two main evolutionary trends in their reproduction (Corbet, 1980).

In Odonata, endophytic oviposition is assumed to be a plesiomorphic feature (Bechly et al., 2001). Females deposit their eggs within plant tissues by means of a well-developed ovipositor

*Email: odonataly@gmail.com

composed of the genitalic appendages of the 8th and 9th abdominal segments. About 54% (c.3000) species of recent dragonflies demonstrate endophytic egg-laying behaviour, including all Zygoptera, Anisozygoptera and anisopteran Aeshnidae (Bridges, 1993). Alternatively, the eggs are either dropped freely into the water or attached superficially onto water plants or other periaquatic objects (i.e. stones, soil, tufts of grasses, pads of roots, etc). This derived egg laying strategy is called exophytic oviposition (Carle et al., 2008). It is practised by Anisoptera with reduced appendicular ovipositors, e.g. Gomphidae, Corduliidae, Libellulidae, Chlorogomphidae, and Neopetaliidae (Carle & Louton, 1994; St. Quentin, 1962). Those taxa are called 'exophytic Odonata' (Corbet, 1999). The exophytically ovipositing taxa include c.43% (c.2400 species) of recent dragonflies (Bridges, 1993). It is possible that exophytic oviposition permits dragonflies to explore new larval habitats, e.g. temporary waters lacking, or with scarce, suitable vegetation (Corbet, 1962, p. 8).

During the last 15 years extensive work has been carried out on the comparative and functional morphology of the plesiomorphic well-developed ovipositor in Odonata. Specific studies were focused on the skeleton and musculature (e.g. Klass, 2008; Matushkina, 2004, 2008a, 2008b; Matushkina & Gorb, 1997; Matushkina & Klass, 2011; Matushkina & Lambret, 2011), cuticular microstructures (e.g. Matushkina, 2008b; Matushkina & Lambret, 2011; Matushkina & Klass, 2011), and functional aspects of the endophytic ovipositor (e.g. Matushkina & Gorb, 2002, 2007; Matushkina & Lambret, 2011; Matushkina & Klass, 2011). Conversely, there is little information on morphology of the reduced-type female genitalia suited for exophytic oviposition. Apart from general views of genitalic segments drawn in original descriptions of the species (e.g. Machado & Costa, 1995), identification keys (e.g. Garrison et al., 2006), and morphobiological reviews (e.g. Tillyard, 1917), more detailed descriptions of female external genitalia and related musculature in exophytically ovipositing dragonflies are lacking. The exception is *Davidius nanus* (Selys, 1869) (Gomphidae), whose female genitalic segments were investigated with special emphasis placed on the skeletal musculature (Asahina, 1954). The cuticle microstructure of the reduced female external genitalia remained unexplored. However, such studies are strongly needed to understand the scenario of reduction (or multiple reductions) of the appendicular ovipositor that may have occurred during dragonfly evolution. A more detailed study would also reveal functional aspects of exophytic egg laying carried out by some of the most successful lineages of recent Odonata.

The present work studies the ovipositor remnants in representatives of the three most species-rich families of exophytically ovipositing Odonata (Gomphidae, Corduliidae, Libellulidae) in order to consider their possible use in oviposition. It is attempted to homologize them with structures of the plesiomorphic well-developed ovipositor.

Materials and methods

Adult females of *Somatochlora metallica*, *Epithecina bimaculata* and *Gomphus vulgatissimus* were collected from Central Ukraine (Cherkassy oblast, vicinities of Kanev Natural Reserve, surroundings of villages Lipyave and Pekari; May–July 1999–2008). A teneral female of *Onychogomphus forcipatus* was kindly provided by Prof. Stanislav N. Gorb from his personal collection. A female of *Lindenia tetraphylla* was obtained from the insect collection of the Zoological Institute of the Russian Academy of Sciences, ZIN (St. Petersburg, Russia). Adult females of other species were collected from Northern Ukraine (Kyiv oblast, Vyshgorodsky district, surroundings of village Khotyanivka; May–October 2000–2010). Before examinations, dried specimens were softened with Bouin's solution for a few months and afterwards with 10% lactic acid for 10–15 min (Table 1). Abdominal segments were dissected in frontal and if possible in median planes. The musculature was examined in water under a stereomicroscope by manual dissections

Table 1. Characteristics of material examined.

Odonata species	Family	Number of specimens and preservation	Examination
<i>Gomphus vulgatissimus</i> (Linnaeus, 1758)	Gomphidae	1dr, 1et	M, LM, SEM
<i>Onychogomphus forcipatus</i> (Linnaeus, 1758)	Gomphidae	1et	M, LM
<i>Lindenia tetraphylla</i> (Vander Linden, 1825)	Gomphidae	2dr	M, LM
<i>Cordulia aenea</i> (Linnaeus, 1758)	Corduliidae	2et, 1B	M, LM, SEM
<i>Epithea bimaculata</i> (Charpentier, 1825)	Corduliidae	1et, 1B	M, LM
<i>Somatochlora metallica</i> (Vander Linden, 1825)	Corduliidae	2B	M, LM
<i>Orthetrum cancellatum</i> (Linnaeus, 1758)	Libellulidae	2et	M, LM
<i>Sympetrum vulgatum</i> (Linnaeus, 1758)	Libellulidae	2et	M, LM
<i>Sympetrum sanguineum</i> (Müller, 1764)	Libellulidae	1et	M, LM

Et, 70% ethanol; dr, dried; B, Bouin's solution; M, muscles; LM, light microscopy; SEM, scanning electron microscopy.

and subsequent layer-by-layer reconstructions of muscles. After that cuticular parts were treated with 10% KOH, washed in water, and examined in glycerine under a stereomicroscope. For scanning electron microscopy, the cuticular parts of two species were washed in distilled water, dehydrated in a graded ethanol series and in acetone, critical point dried (OM CPD 7501), sputtered with gold-palladium (OM-SC7640) and examined with a Zeiss EVO-50 SEM. In all, nine species were investigated by means of light microscopy to describe the ovipositor-related musculature and cuticle structures of genitalic segments. Two species were additionally studied with the scanning electron microscopy (SEM) to reveal cuticular microstructures situated on genitalic appendages.

Abdominal musculature was compared to Asahina (1954) and Matushkina & Gorb (1997). Abbreviations: vl, vulvar lamina (valvula vulvae, vulvar scale, subgenital plate); r, rudiments on the 9th sternum (vestigis, stylets); S8, S9, S10, abdominal segments 8, 9 and 10, respectively; M4, M7, muscles of ovipositor.

Results

General remarks

The vulvar lamina is a paired or unpaired plate of variable shape and size located on the posterior margin of the 8th sternite (Figure 1). It is unmovable relative to the 8th sternite in all species studied in this regard. The rudiments on 9th sternite (occasionally absent) are knob- to rod-shaped appendages located in the anterior half or near the middle of the 9th sternum. Of the ovipositor-related muscles, only paired M4 and unpaired M7 are found (Figure 2a–c). The origin of M4 is located on the 9th tergite, and insertion is on the 9th sternum lateral to the rudiments. M7 is attached to the 9th sternum beside the rudiments (laterally or dorsolaterally to them), always behind the attachment points of M4 (Figure 2d–f).

Gomphidae

Gomphus vulgatissimus

The vulvar lamina is unpaired, with a deep V-shaped apical incision with acute divergent lobes (Figure 3a, b). Sensilla of two morphotypes are scattered on the lobes. The styloconic sensilla

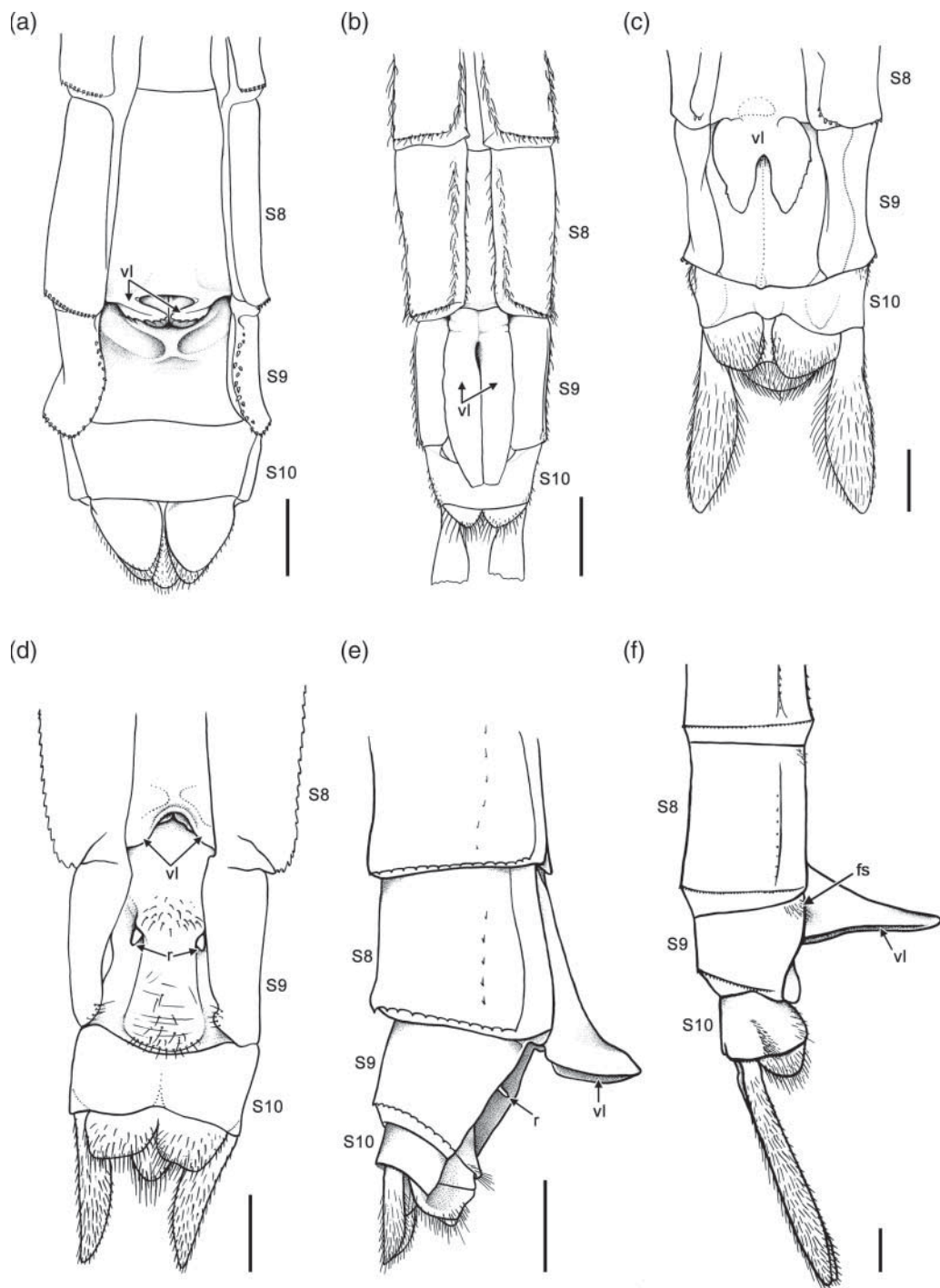


Figure 1. The female terminal segments of some exophytically ovipositing Odonata, ventral (a–d) and lateral (e, f) views: (a) *Onychogomphus forcipatus* (Gomphidae); (b) *Epithea bimaculata* (Corduliidae); (c) *Cordulia aenea* (Corduliidae); (d) *Orthetrum cancellatum* (Libellulidae); (e) *Sympetrum vulgatum* (Libellulidae); (f) *Somatochlora metallica* (Corduliidae). Cerci in (b) are cut off. fs, field of setae; r, rudiments on the 9th sternum; S8, S9, S10, abdominal segments 8, 9 and 10, respectively; vl, vulvar lamina. Scale bars = 1 mm.

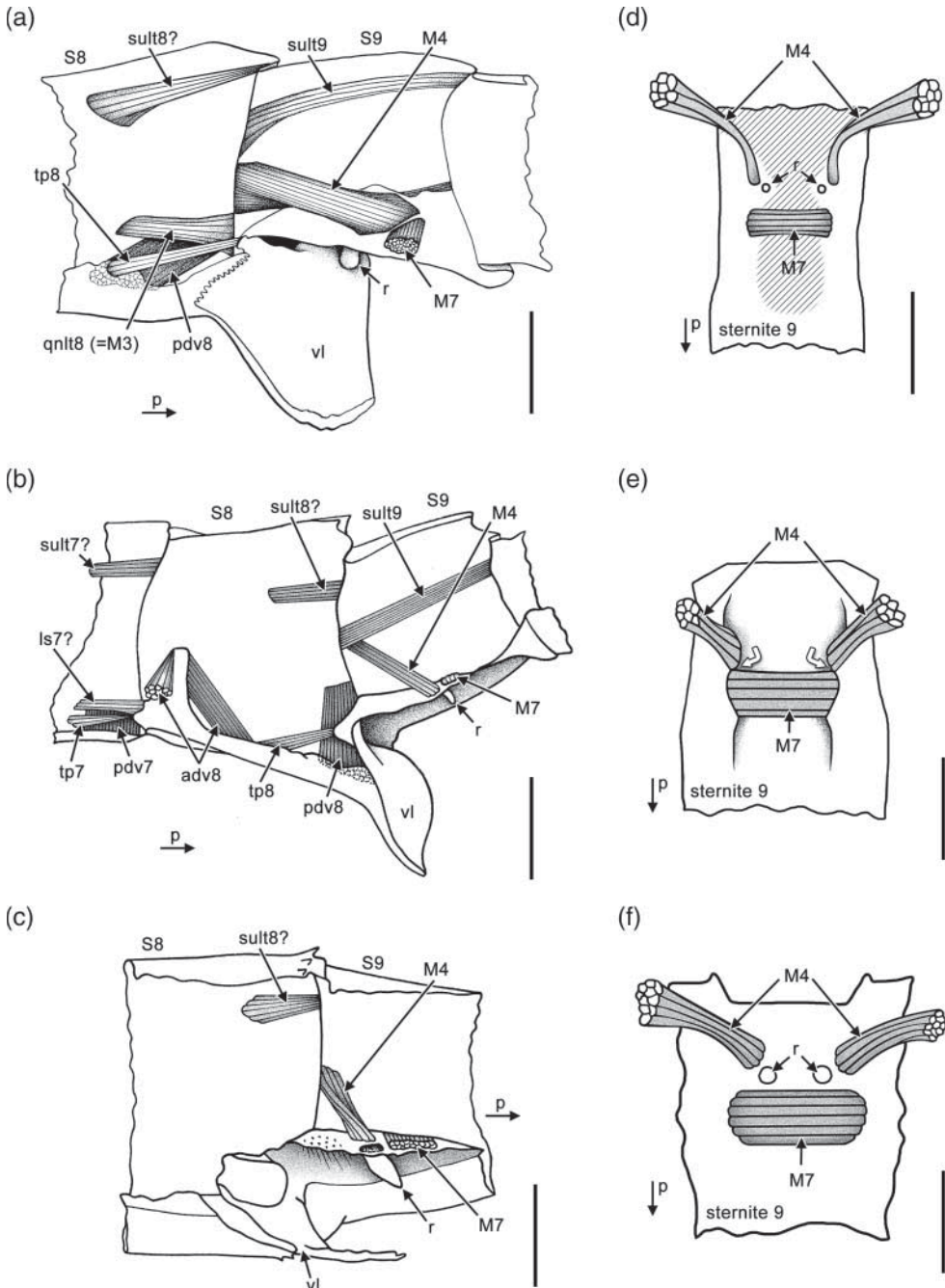


Figure 2. The muscle arrangement of the female genitalic segments in lateral view (a–c), and diagrammatic internal views of the 9th sternum showing position of the attachment points of the ovipositor muscles relative to the rudiments (d–f), skeletal muscles not associated with the ovipositor derivatives are partly removed: (a) *Somatochlora metallica* (Corduliidae); (b) *Sympetrum vulgatum* (Libellulidae); (c), (f) *Cordulia aenea* (Corduliidae); (d) *Epiheca bimaculata* (Corduliidae); (e) *Orthetrum cancellatum* (Libellulidae). White arrows in (e) indicate position of rudiments. Membranous part of the 9th sternum in (d) is hatched. Posterior margins of the 9th sternum in (d–f) are not shown. M4, M7, muscles of ovipositor; p, posterior direction; r, rudiments on the 9th sternum; S8, S9, abdominal segments 8 and 9, respectively; vi, vulvar lamina; abdominal muscles of corresponding segment (number): adv, anterior dorsoventral muscle; ls, longitudinal sternal muscle; pdv, posterior dorsoventral muscle; qnl8, quinary longitudinal tergal muscle; sult, superior longitudinal tergal muscle; tp, tergopleural muscle. Scale bars = 1 mm.

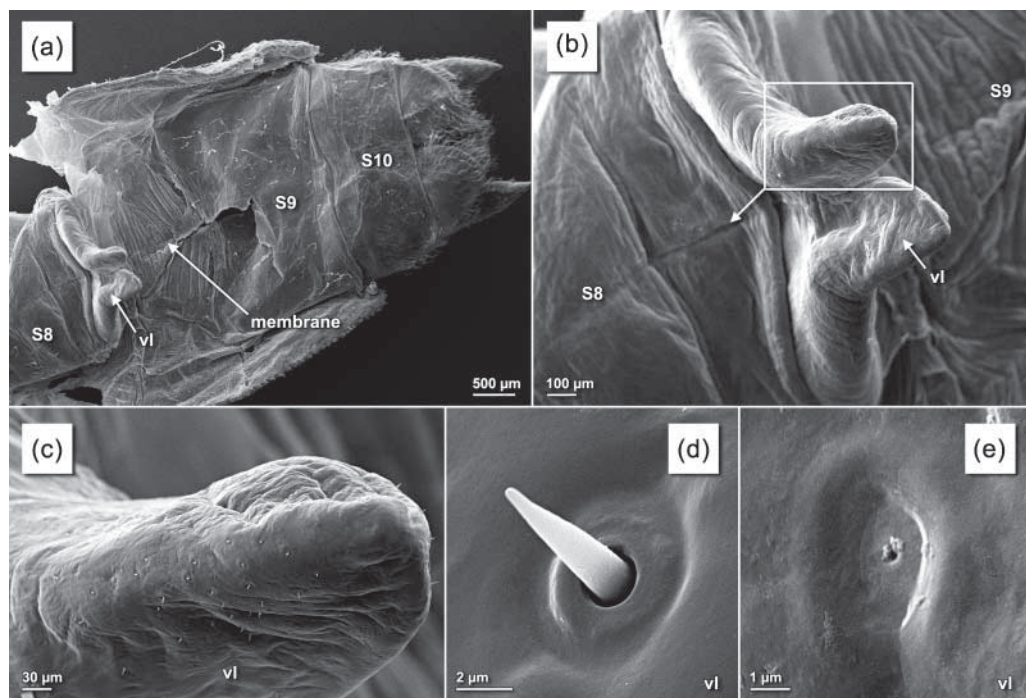


Figure 3. SEM micrographs of female genitalic segments of *Gomphus vulgatissimus* (Gomphidae), ventral view: (a) general aspects; (b) vulvar lamina; (c) lobe of vulvar lamina showing distribution of sensilla; (d) styloconic sensillum of vulvar lamina; (e) campaniform sensillum of vulvar lamina. Inset in (b) enlarged in (c). S8, S9, S10, abdominal segments 8, 9 and 10, respectively; vl, vulvar lamina.

$c.6\text{--}7.5\text{ }\mu\text{m}$ in length are numerous, slightly aggregated on the anterolateral surface of the lobe (Figure 3c, d). The campaniform sensilla, $c.2.5\text{--}3\text{ }\mu\text{m}$ in length, are very rare; they are scattered randomly over the anterior part of the lobe (Figure 3e). The 9th sternum consists of a depressed membranous anterior part and a bulging sclerotized posterior part. Both the rudiments and ovipositor-related muscles are absent.

Onychogomphus forcipatus

The paired vulvar lamina consists of two separated lobes that are convergent medially at their apices (Figure 1a). The posterior edge of each lobe is slightly serrated and bears long setae. There are swollen walls of the genital openings above the lobes. The anterior part of the 9th sternum is membranous. The sclerotized posterior part of the 9th sternum has bilateral oblique lateral depressions and a round medial depression in its anterior half. The rudiments and ovipositor-related muscles are absent. The specimen studied was apparently teneral.

Lindenia tetraphylla

The vulvar lamina is short, unpaired, with subtriangular lobes in ventral aspect. The entire 9th sternum is poorly sclerotized; its posterior part is softer but not entirely membranous. The rudiments and ovipositor-related muscles are absent. The lateral walls of the 8th and 9th sterna are covered on the side with the wing-shaped dilatations of the 7th, 8th and 9th tergites.

Corduliidae

Epitheca bimaculata

The vulvar lamina is unpaired, with very long lateral lobes that are fused basally and conspicuously less sclerotized than surrounding sclerites. It runs parallel to the 9th sternum in lateral view and extends far beyond the 9th segment (Figure 1b). The 9th sternum is weakly sclerotized medially and anteriorly, appearing as a U-shaped sclerite surrounded by membrane. This shape allows the sclerite to bend along the median line in a transverse plane. The rudiments are knob-shaped, located on the sclerite at about the middle of the 9th sternum. They may touch the dorsal wall of the vulvar lamina. Anterior parts of the 9th sternite bear paired fields of long setae in front of the rudiments. Attachment points of the muscles M4 and M7 are located on sclerotized parts of the 9th sternum lateral to the rudiments (Figure 2d).

Cordulia aenea

The vulvar lamina is nearly half as long as the 9th sternum, directed parallel to the abdomen. It is an unpaired plate with a V-shaped median cleft for about half the length of the lamina (Figure 1c). The subtriangular lobes with numerous denticles, styloconic sensilla (from 10 to 17 μm in length) and campaniform sensilla ($c.4 \mu\text{m}$ in length) that are scattered on the ventral and dorsoapical surfaces (Figure 4b, c, g). The 9th sternum is evenly sclerotized, with nipple-shaped rudiments at the middle region (Figure 4a, e). There are three morphotypes of sensilla on the lateroapical parts of each rudiment (Figure 4f). Styloconic sensilla and campaniform sensilla ($c.4 \mu\text{m}$ in length) are very sparse, situated mostly on the middle part of the rudiment. Basiconic sensilla are notably more numerous, aggregated apically (Figure 4e, f). Each basiconic sensillum has corrugated surfaces on the basal collar ($c.8 \mu\text{m}$ across diameter) and setae ($c.3\text{--}4 \mu\text{m}$ in length), and bears a single, apical pore (Figure 4h). During extrusion of the 9th sternum ventrally, the rudiments touch the dorsal surface of vulvar lamina. Thin setae of different length (from 60 μm to 220 μm long) are aggregated in a field on the 9th sternite situated between the rudiments and anterior margin of the sternite (Figure 4a, d). Muscles M4 and M7 are present (Figure 2c, f).

Somatochlora metallica

The vulvar lamina is unpaired, acutely pointed, as long as the 9th and 10th sterna, directed almost perpendicular to the abdomen (Figure 1f). The lamina is broad at the base, its anterolateral edges overlie by the anterolateral edges of the 9th tergite, which has a paired field of long setae on this place. The 9th sternum is evenly sclerotized. The rudiments are spherical; they may touch the dorsal surface of the vulvar lamina. There are fields of long setae on the 9th sternite in front of the rudiments. Muscles M4 and M7 are broad, attached to the 9th sternite posteriorly to the rudiments (Figure 2a).

Libellulidae

Orthetrum cancellatum

The vulvar lamina is paired and consists of two short square lobes bearing short setae (Figure 1d). The anterior part of the 9th sternum is less sclerotized than its remaining surface. The 9th sternite is distinct convex at the mid-line. The rudiments are nipple-shaped; they are attached to the

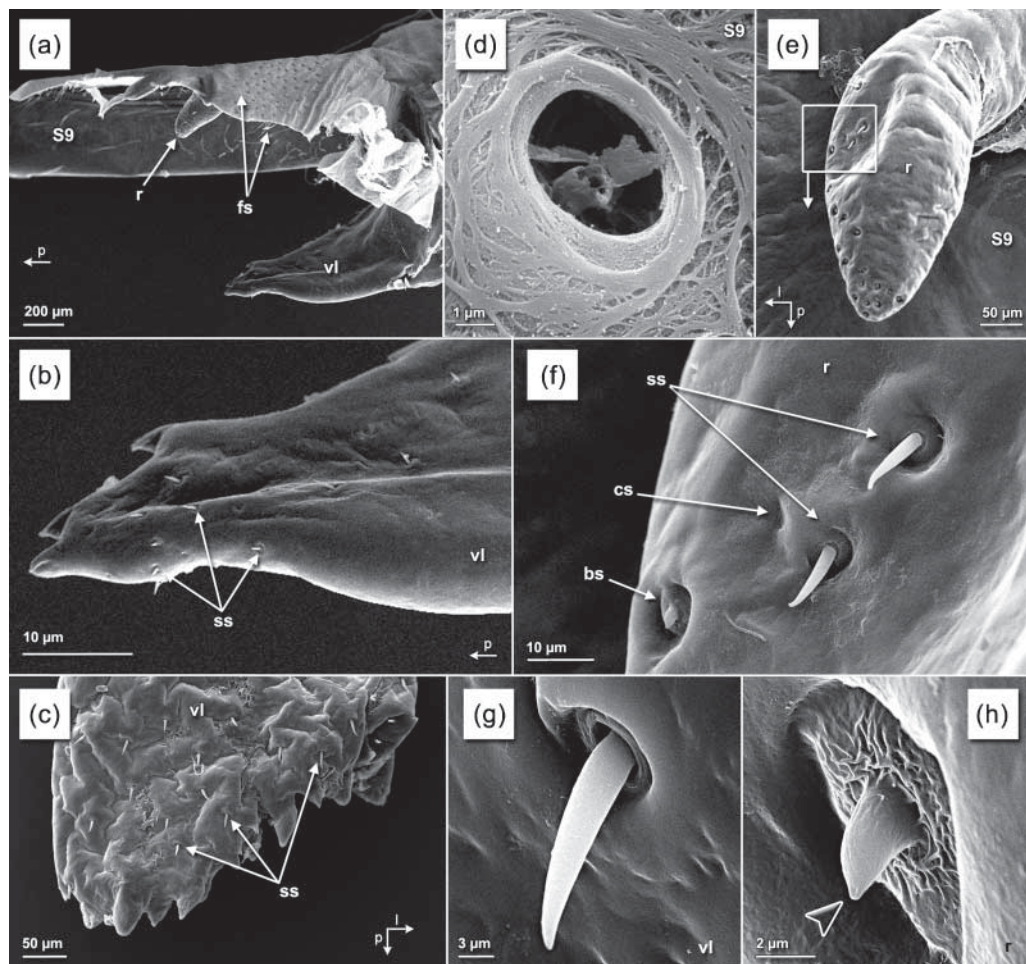


Figure 4. SEM micrographs of female genital segments of *Cordulia aenea* (Corduliidae): (a) ovipositor derivatives in median view (9th sternite is partly turned inside out to show the density of setae in front of rudiments); (b) lobe of vulvar lamina in median view; (c) lobe of vulvar lamina with scattered styloconic sensilla in ventral view; (d) underside surface of single seta (trichoid sensillum?) from the field of setae in front of rudiments; (e) rudiment in ventral view showing distribution of sensilla; (f) sensillum types of rudiment; (g) styloconic sensillum of vulvar lamina; (h) basiconic sensillum of rudiment bearing a single, apical pore indicated with an arrowhead. Inset in (e) enlarged in (f). bs, basiconic sensillum; cs, campaniform sensillum; fs, field of setae; l, lateral direction; p, posterior direction; r, rudiment on the 9th sternum; ss, styloconic sensillum; S9, abdominal segment 9; vl, vulvar lamina.

9th sternite beside its convex region. Muscles M4 and M7 are broad. Attachment points of M7 are situated on the upright wall of the 9th sternum dorsally to the rudiments. M4 is attached to the 9th sternum in front of M7 (Figure 2e).

Sympetrum vulgatum

The vulvar lamina is unpaired, upright, with apex slightly bent posteriorly. The 9th sternum is entirely sclerotized. The rudiments are rod-shaped (Figure 1e). The muscles M4 and M7 are broad, attached to the 9th sternite laterally to the rudiments (Figure 2b). When the 9th sternite is extruded ventrally, the rudiments touch the pleural membrane between tergite and sternite.

Sympetrum sanguineum

The unpaired vulvar lamina is short, rounded, with a small apical incision. The 9th sternum is entirely sclerotized, without any rudiments. Muscles M4 and M7 are broad, attached to the 9th sternite nearly at its middle part, much as in *S. vulgatum*.

Discussion

Reduction of the ovipositor in exophytically ovipositing Odonata: what exactly remains?

It is generally accepted that the evolution of Gomphidae and Libelluloidea (including Corduliidae and Libellulidae) was accompanied by a reduction of the plesiomorphic well-developed ovipositor and by associated changes in oviposition behaviour (St. Quentin, 1962). The well-developed ovipositor in Odonata represents three main elements: (1) the shaft of the ovipositor, including paired cutting 1st and 2nd valves (gonapophyses of 8th and 9th segments, respectively) suitable for substrate penetration; (2) paired large plates, the 3rd valves (gonocoxites of 9th segment, the gonopla IX following the terminology of Klass, 2008) which ensheath the shaft of the ovipositor in its resting position; the distal edges of the 3rd valves bear moveable stick-like appendages, the styli (gonostyli of 9th segment), which have been identified as sensory structures (Matushkina & Gorb, 2002); (3) several sclerites associated with the ovipositor valves (paired gonocoxites of 8th segment and gonanguli, unpaired internal sclerite) (e.g. Asahina, 1954; Klass, 2008; Matushkina, 2004, 2008a, 2008b; Matushkina & Gorb, 1997; Pfau, 1985). The complete muscle set of the endophytic ovipositor comprises seven paired muscles (M1–6, M8) and one unpaired muscle (M7) in Zygoptera and Anisozygoptera (Matushkina & Gorb, 1997), whereas in aeshnid Anisoptera one paired muscle (M6) is lost (Asahina, 1954; Pfau, 1991; Matushkina & Gorb, 1997, Matushkina, 2008a). In exophytically ovipositing Odonata, the remnants of the ovipositor are represented by the vulvar lamina and, usually, by a pair of rudimentary on 9th sternite (Figures 1, 3a, 4a) and by ovipositor muscles M4 and M7 (Figure 2).

The origin of the ovipositor components in hemimetabolous insects may be ascertained by tracing their development during postembryogenesis (Machotin, 1929, 1953). In dragonflies, the rudiments of the external genitalia appear on larval stages and enlarge gradually instar-by-instar (van der Weele, 1906; Machotin, 1929; Asahina, 1954; Matushkina, 2008a). In particular, in early instars of the aeshnid, *Aeshna grandis* (Linnaeus, 1758), two pairs of outgrowths appear on the ventral surface of the genitalic segments at first. Those of the 8th segment become the 1st valves of the adult ovipositor (8th gonapophyses) and those of the ninth become the 2nd valves (9th gonapophyses). The outgrowths on the 9th sternum develop in association with basal plates (9th gonocoxites), which become enlarged in the form of processes in the next larval stages. The styli become detached from the distal parts of the processes (9th gonocoxites) subsequently (Machotin, 1929). Thus the final aeshnid instar exhibits three paired valves and styli, all as the unmovable rudiments, and has three ovipositor muscles, paired M4 and M5, and unpaired M7. Final development of the ovipositor components occurs during maturation of the female (Matushkina, 2008a).

The development pattern of the exophytic ovipositor is basically similar throughout the Odonata studied here (see e.g. Machotin, 1929; van der Weele, 1906). During larval development the ovipositor rudiments in corduliids arise as paired small knobs at the hind margin of the 8th sternum (the rudiments of vulvar lamina) and paired plates in the middle part of the 9th sternum (van der Weele, 1906). In larval gomphids, the rudiments of female external genitalia are usually absent (van der Weele, 1906); only the final larvae of *Erpetogomphus designatus* Hagen in Selys, 1858 (Hagen, 1885, cited after van der Weele, 1906) and *Stylurus flavipes* (Charpentier, 1825)

(Machotin, 1929) possess rudiments of the vulvar lamina in form of two cylindrical knobs, which can be covered with hind margin of the 8th sternite (Suhling & Müller, 1996). The larval libellulids exhibit almost no sign of the external genitalia rudiments (Machotin, 1929; Gerken & Sternberg, 1999). Rudiment size in a larva is assumed to be not correlated with a size of the external genitalia in adult females (Machotin, 1929).

There is still disagreement concerning homology of certain of the ovipositor remnants in exophytically ovipositing Odonata. The vulvar lamina is recognized as the remnant of the 8th gonapophyses (apparently fused with 8th gonocoxites) that are fused along their median line to a variable extent and connected immovably to the 8th sternite (Asahina, 1954; Machotin, 1929; St. Quentin, 1962; van der Weele, 1906), or is considered to be a new formation of the 8th sternum (Ris, 1909–1916, cited after St. Quentin, 1962). The origin of the rudiments on the 9th sternum in exophytically ovipositing Odonata is a matter of long-standing contention. It was assumed that the rudiments are derived from the styli of the 9th segment (Machotin, 1929; Miller, 1989), based on superficial similarity and sensory function shared by both appendages. In contrast, the rudiments were considered as remnants of the 9th gonapophyses (St. Quentin, 1962; Tillyard, 1917; van der Weele, 1906), based on topographic similarity between the developing 9th gonapophyses in aeshnid larvae and rudiments in imagos of exophytically ovipositing dragonflies.

The reduction of the ovipositor in Odonata could be convincingly illustrated in terms of the concept of heterochrony, i.e. an evolution by “changes in developmental timing that produce parallels between the stages of ontogeny and phylogeny” (Gould, 1977, p. 2, cited after Vinicius & Lahr, 2003). The heterochrony appears as retardation or acceleration of growth and development of body parts and provides a widespread mechanism of the morphological changes of animals during their evolution (Matsuda, 1987). In insects, heterochrony has been intensively investigated in regard to the evolution of sociality (e.g. Linksvayer & Wade, 2005; Nalepa & Bandi, 2000) as well as to the morphological novelties of termitophilous species (e.g. Disney, 1997). If heterochronic development occurred in female external genitalia of exophytically ovipositing Odonata, it is to be expected that the reduced adult genitalia bear similarities (and homologies) with those of larval stage(s) of presumably more plesiomorphic aeshnid dragonflies. Ovipositor rudiments in aeshnid larvae are superficially similar to the remnants of the ovipositor in adult of exophytically ovipositing Odonata (compare figures 30, 31 in van der Weele, 1906 and Figure 1 in this study). The rudiments on the 9th sternum found in aeshnid larvae appear to be homologous with those in adult female of exophytically ovipositing taxa and represent developed 9th gonapophyses. In other words, if there is only one pair of rudiments on the 9th sternum in the female of an exophytically ovipositing dragonfly, they are rather the rudimentary 9th gonapophyses than the styli.

This presumption receives some additional support by the results of this study. As previously mentioned, the rudiments on the 9th sternum of the corduliid, *Cordulia aenea*, are richly covered by sensilla of different morphotypes (Figure 4e, f, h). The styloconic and campaniform sensilla were found both on the vulvar laminae of exophytically ovipositing Odonata and on three paired valves and styli of the endophytic ovipositor (e.g. in *Epiophlebia superstes* (Selys, 1889) by Matushkina, 2008b, petalurid anisopteran *Phenes raptor* Rambur, 1842 by Matushkina & Klass, 2011, and lepidopteran *Lestes macrostigma* (Eversmann, 1836) by Matushkina & Lambret, 2011). In contrast, the basiconic sensilla bearing a single apical pore are morphologically similar to those found on the gonapophyses of the well-developed ovipositor, such as in *P. raptor* (Matushkina & Klass, 2011), *L. macrostigma* (Matushkina & Lambret, 2011), and in several aeshnids of the genera *Aeshna* and *Anax* (Matushkina, unpubl. data). Additional support may be found in the muscle arrangements of the 9th segment. The relative position of attachment points of the ovipositor muscles M4 and M7 and the rudiments on 9th sternum in exophytically ovipositing Odonata is constant (Figure 2). Moreover this region is topographically identical to that in the final instar of *Anax imperator* Leach, 1815 where attachment points of M4 and M7 and the rudiments of the 9th gonapophyses are situated (Matushkina, 2008a, figure 1b).

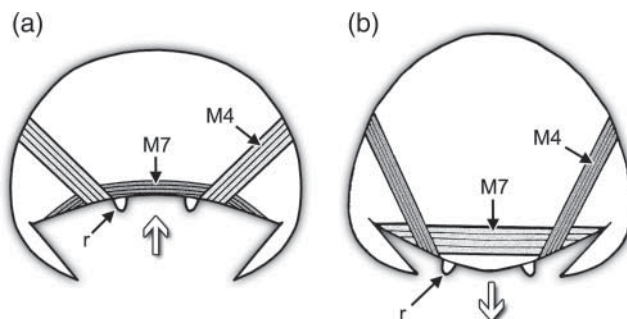


Figure 5. Diagrams illustrating the suggested antagonistic functioning of the ovipositor muscles M4 and M7 in females of exophytically ovipositing Odonata, cross section of 9th segment: (a) contraction of the paired muscle M4 causes upward movement of the middle part of the 9th sternum with rudiments; (b) antagonistic contraction of the unpaired muscle M7 draws the lateral parts of 9th sternum together resulting a downward movement of the middle part the 9th sternum where rudiments are usually located. Note that volume of the 9th segment changes as a result of contractions of ovipositor muscles. White arrows indicate upward and downward movements of the middle part of the 9th sternum with rudiments. M4, M7, muscles of ovipositor; r, rudiment on the 9th sternum.

Morphofunctional interpretation of ovipositor remnants

It appears to be most likely that remnants of ovipositor-related muscles, M4 and M7, are functioning antagonistically (Figure 5). Contraction of the paired muscle M4 leads to upward movement of the middle part of the 9th sternum. As a result, an area behind the genital opening between the vulvar lamina and the 9th sternum expands. The eggs can be released both synchronously and asynchronously with a retraction of the 9th sternum, as it was observed during artificial ovipositions carried out by captured females of several libellulid dragonflies (Matushkina, unpubl. data). Antagonistic contraction of the unpaired muscle M7 draws the lateral parts of 9th sternum together, i.e. toward the middle line. This causes a downward movement of the middle part the 9th sternum during which the sternum is bent in a transverse plane. Especially *Epithecina bimaculata* (Corduliidae) exhibits a membranous middle strip at the 9th sternum that facilitates transverse bending. Since the rudiments are located at this place in most studied species, as a result of contraction of M7 the rudiments make contact with either the vulvar lamina (all studied corduliids) or lateral walls of the 9th segment (*Sympetrum vulgatum*). In any case, the transverse bending of the 9th sternum leads to reduction or even closure of the free area behind the genital opening bounded by the vulvar lamina ventrally and 9th sternum dorsally. Antagonistic contraction of the ovipositor muscles described above leads simultaneously to changes in volume of the 9th segment.

In gomphids, the 9th sternum is sclerotized differently. The most anterior part of the 9th sternum represents a weakly sclerotized or membranous area that is moveable in the dorsoventral direction. The posterior part of the 9th sternum is entirely sclerotized, often forming a bulging sclerite that can hardly bend in the transverse plane. The peculiar sclerotization of the 9th sternum was emphasized by van der Weele (1906, p. 177) as well. The gomphid dragonflies also characteristically lack the rudiments on 9th sternum, as found for example in *Davidius nanus* (Asahina, 1954), though the presence of a paired hairy plate in the middle part of 9th sternum in *Onychogomphus uncatius* (Charpentier, 1840) was recorded (St. Quentin, 1962). It appears to be likely that the described peculiarities of the sclerotization of the 9th sternum correlate with the absence of ovipositor muscles.

In two species (*G. vulgatissimus*, *C. aenea*) both the vulvar lamina and rudiments are richly equipped with sensilla (Figures 3, 4). Styloconic sensilla represent the most abundant type present on the external genitalia of both species studied. Numerous styloconic sensilla are

characteristically situated on the vulvar lamina tending to aggregate slightly on its lateroapical and ventral parts (Figures 3c, d, 4b, c, g). Because of the smooth surface of the sensillar setae and absence of any visible pore, the styloconic sensilla most likely cannot be considered as chemosensory detectors (Zacharuk, 1980). Campaniform sensilla are much rarer and randomly scattered among the styloconic ones on the vulvar lamina (Figure 3e). This sensillum morphotype characteristically serves a mechanosensory function in insects detecting the stress and strains arisen in cuticle during its deformation (Ivanov, 2000). A few styloconic and campaniform sensilla are also present on the rudiments on the 9th sternum in *C. aenea* (Figure 4e, f). Basiconic sensilla with a single terminal pore were found only on the rudiments of the 9th sternum in *C. aenea* where they are situated on the distal half and are more numerous apically (Figure 4e, f, h). The presence of a pore in the sensillum seta implies that the basiconic sensilla are not exclusively mechanoreceptive, if at all, and probably can perceive some chemical stimuli (Zacharuk, 1980). In several species studied, the region between the genital opening and rudiments bears a(some) field(s) of setae, which may be composed of trichoid sensilla.

Behavioural aspects: how might the ovipositor-related structure affect reproduction?

In exophytically ovipositing Odonata, the female external genitalia can be used in two main processes of reproduction: mating and egg laying, where the latter function is understood to include oviposition site selection as well as egg laying itself.

In contrast to other stages of the reproduction, mating is well investigated with respect to the functioning of the female genitalia in exophytically ovipositing Odonata. In particular, the presence of a median cleft or deep incision in the extended vulvar lamina is correlated with the relatively short penis and long hamules in the male genitalia (recorded for several libellulids by Miller, 1989). Moreover, high morphological variation of the vulvar lamina is assumed to form mating barriers between sympatric species or play a role in sperm competition (Watson, 1966, cited after Miller, 1989). Components of the female and male genitalia can interact either mechanically, e.g. as a key-and-lock device, or through the excitation of particular groups of tactile receptors on the female (Loibl, 1958; Paulson, 1969; Robertson & Paterson, 1982; Tennessen, 1982; all cited after Miller, 1989). In exophytically ovipositing Odonata the styloconic sensilla situated on the vulvar lamina and/or a field of setae located behind the genital opening could function as these tactile receptors.

Recognition of suitable places for oviposition is a complex behavioural reaction of a dragonfly to different stimuli including visual, tactile, thermal and perhaps olfactory ones (Wildermuth, 1994). The main behavioural characteristics of exophytic oviposition that are directly associated with usage of external genitalia are: (1) the eggs are laid by a solitary female or in contact with or under the distant guarding of a male; this means that the male might or might not directly participate in the oviposition site selection; (2) the female oviposits in flight or while sitting on the substrate (usually vegetation); (3) the eggs are released singly, as egg strings and other small clusters, or in form of a large egg mass; (4) oviposition occurs onto/into the water, vegetation or soil (Beckemeyer, 2004; Corbet, 1962; Schiemenz, 1953; Schorr, 1990; Suhling & Müller, 1996; Wildermuth, 1994). These peculiarities of oviposition behaviour characterize some of the taxa of exophytically ovipositing Odonata, although interspecific, intraspecific and intra-individual variation in oviposition behaviours occur (Corbet, 1980; Paulson, 1969; Rowe, 1988). Libellulid females, in particular, typically scatter their eggs in flight by making intermittent contact with the water surface. Prior to water contact of the abdomen the eggs accumulate under the 9th abdominal sternite on the vulvar lamina, forming a small cluster before they are washed off into the water (Miller, 1987; Andrew & Tembhare, 1996; all cited after Gaino et al., 2008). For several species of Libellulinae it is thought that the lateral flaps of the female genitalic segments are

Table 2. Ovipositor morphology and egg-laying behaviour in studied exophytically ovipositing Odonata.

Species	Ovipositor morphology					Oviposition					Literature/Notes
	vl size class	vl shape	vl position	presence of rudiments	presence of muscles	male contact guarding	pose of female	contact with substrate	substrate type	egg clutch	
<i>Gomphus vulgatissimus</i>	II	sl	t	—	—	—	s, f	+	w	s, cl	Schiemenz, 1953; Suhling & Müller, 1996
<i>Onychogomphus forcipatus</i>	II	sl	t	—	—	—	f	+	w	?	Schiemenz, 1953; Suhling & Müller, 1996
<i>Lindenia tetraphylla</i>	II	sl	p	—	—	—?	?	?	?	?	no information on oviposition (Schorr et al., 1998)
<i>Cordulia aenea</i>	III	f	p	+	+	—	s, f	+	w	cl	Schiemenz, 1953; original observations
<i>Epithea bimaculata</i>	III	sl	p	+	+	—	s, f	+	w	cl	Schiemenz, 1953; original observations
<i>Somatochlora metallica</i>	III	f	t	+	+	—	f	+	w, s, v	s	Schiemenz, 1953; Fox, 1991
<i>Orthetrum cancellatum</i>	I	sl	p	+	+	—	f	+	w	cl	Schiemenz, 1953
<i>Sympetrum vulgatum</i>	III	f	t	+, —	+	+	f	+, —	w, s	s	Schiemenz, 1953
<i>Sympetrum sanguineum</i>	II	f	t	—	+	+	f	—	w, s	s	Schiemenz, 1953; Miller, 1989

Notes: Size class of vl (after Miller, 1989): I – vl is either not extended posteriorly, or is extended for 10% of the length of S9; II – vl extending for 10–33% of the length of S9; or III – vl extending for more than 33% of the length of S9. Shape of vl: sl, slotted; f, fused. Position of vl (relative to S9): t, turned ventrally; p, running almost parallel to S9. The + and — symbols indicate presence and absence of: rudiments; (ovipositor) muscles; male contact guarding (tandem oviposition); and contact with substrate (by female genitalia during oviposition). Pose of (ovipositing) female: f, flying, s, sitting. Substrate type (in/on which eggs can be released): w, water, s, soil, v, vegetation. Egg clutch: cl, egg cluster, s, single egg; ?, unknown or uncertain information.

associated with the so-called scooping oviposition wherein the female “scoop[s] the droplets of water from the surface and fling[s] them (presumably with eggs washed of the abdomen and carried in the droplets) onto plants or other nearby substrates” (Beckemeyer, 2004). Females of several gomphids, prior to laying, apparently accumulated and held eggs under the 9th sternum using the vulvar lamina and dilated tergites of genitalic segments (van der Weele, 1906).

Available literature reveals a certain behavioural flexibility of egg laying tactics shared by the species studied here (Table 2; see references therein). They all preferably oviposit in flight, with the exception of the gomphid *G. vulgatissimus*, and two corduliids *C. aenea* and *E. bimaculata*, which may also lay eggs while sitting on vegetation. Apart from *Sympetrum* species that usually oviposit in a tandem, females of the remaining dragonflies studied in this study characteristically oviposit alone (this particularly is the case in Gomphidae). The eggs are laid one by one (*G. vulgatissimus*, *S. metallica*, several species of *Sympetrum*) or in clusters (*G. vulgatissimus*, *C. aenea*, *E. bimaculata*, *O. cancellatum*). Those females most often perform characteristic movements of the abdomen by swinging the tip down and washing the egg(s) from the genital opening or vulvar lamina by means of physical contact with the water. But for some species (e.g. the libellulid *S. sanguineum*, the gomphid *Davidius*) that also oviposit over dry soil or drop eggs high above the water surface, another egg-releasing mechanism must be assumed (Eda, 1960, cited after Corbet, 1962; Radford, 1995). In particular, the corduliid, *S. metallica*, often does not make contact with the water but pushes the eggs into soil, *Sphagnum* moss and peat, or mats of floating vegetation by means of its extended vulvar lamina (Fox, 1991; Schiemenz, 1953). In several corduliid and libellulid species, extended and ventrally pointed vulvar laminae are associated with a specialized mode of oviposition, like pushing eggs into the mud or epiphytic oviposition (Miller, 1989; see also Table III therein), or may hold the accumulating eggs between successive dips to the water (Robert, 1958, cited after Miller, 1989).

The mechanism of egg expulsion in exophytically ovipositing Odonata has not been specifically explored to date. It appears to be an automatic process to a certain degree, since many dragonflies, especially Libellulidae, are known to continue to squeeze out eggs if they are caught during egg laying (Suhling & Müller, 1996). However, the gomphid *O. forcipatus* (as well as *O. uncatus* (Charpentier, 1840) and perhaps the libellulid *Tholymis tillarga* (Fabricius, 1798)) is able to stop the egg delivery after being caught (Suhling & Müller, 1996).

How the ovipositor remnants may be involved in egg delivery, if at all, is still poorly understood and requires further study. Based on the present results, it is logical to assume that downward movement of the 9th sternum can bring the rudiments, richly furnished with sensilla, into contact, either with egg(s) extruded from the genital opening, or with the relatively extended vulvar lamina, or with the male genitalia during mating. As already noted the gomphid dragonflies so far studied are totally different from other species in that they lack ovipositor musculature and rudiments on the 9th sternum. Additionally, successive stages of rudiment reduction, from fully developed to almost absent, were recorded in different specimens of the libellulid species *S. vulgatum* (Machotin, 1929). However, so far as it is known, there are no obvious differences in egg laying behaviour of gomphid dragonflies as compared with other exophytically ovipositing Odonata. Most likely the eggs can be pushed out by means of contractions of muscles situated around the genital opening and/or because of volume changes of 9th segment that occurred during contraction of ovipositor muscles, though some other components of the internal genitalia, like vaginal musculature, may also be involved in egg delivery.

Acknowledgements

The author is grateful to Roy J. Beckemeyer (USA) and two anonymous reviewers for their valuable comments and linguistic help. Specimens were kindly provided by Stanislav N. Gorb (Christian-Albrechts-University of Kiel, Germany) and Viktor A. Krivokhatskiy (Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia).

References

- Asahina, S. (1954). *A morphological study of a relict dragonfly Epiophlebia superstes Selys* (Odonata, Anisozygoptera). Tokyo, Japan Society for the Promotion of Science, pp. 1–153.
- Bechly, G., Brauckmann, C., Zessin, W., & Gröning, E. (2001). New results concerning the morphology of the most ancient dragonflies (Insecta: Odonatoptera) from the Namurian of Hagen-Vorhalle (Germany). *Journal of Zoological Systematics and Evolutionary Research* 39, 209–226.
- Beckemeyer, R.J. (2004). Notes on the behavior and mechanics of scooping oviposition in *Libellula composita* (Hagen) (Anisoptera: Libellulidae). *Odonatologica*, 33, 11–23.
- Bridges, C.A. (1993). *Catalogue of the family-group, genus-group and species-group names of the Odonata of the world* (2nd ed.). Urbana, Illinois: C. A. Bridges.
- Carle, F.L., Kjer, K.M., & May, M.L. (2008). Evolution of Odonata, with special reference to Coenagrionoidea (Zygoptera). *Arthropod Systematics & Phylogeny*, 66, 37–44.
- Carle, F.L. & Louton, J.A. (1994). The larva of *Neopetalia punctata* and establishment of Austropetaliidae fam. nov. (Odonata). *Proceedings of the Entomological Society of Washington*, 96(1), 147–155.
- Corbet, P.S. (1962). *A Biology of Dragonflies*. London, Witherby.
- Corbet, P.S. (1980). Biology of Odonata. *Annual Review of Entomology*, 25, 189–217.
- Corbet, P.S. (1999). *Dragonflies: Behavior and Ecology of Odonata*. Ithaca. New York: Comstock Publishing Associates, Cornell University Press.
- Disney, R.H.L. (1997). Post-eclosion heterochrony in the maturation of the adult females of a termitophilous fly (Diptera, Phoridae). *Bonner Zoologische Beitrage*, 47, 77–86.
- Fox, A.D. (1991). How common is terrestrial oviposition in *Somatochlora metallica* Vander Linden? *Journal of the British Dragonfly Society*, 7(2), 38–39.
- Gaino, E., Piersanti, S., & Rebora, M. (2008). Egg envelope synthesis and chorion modification after oviposition in the dragonfly *Libellula depressa* (Odonata, Libellulidae). *Tissue Cell*, 40, 317–324.
- Garrison, R.W., von Ellenrieder, N., & Louton, J.A. (2006). *Dragonfly genera of the New World: an illustrated and annotated key to the Anisoptera*. Baltimore: Johns Hopkins University Press.
- Gerken, B. & Sternberg, K. (1999). *Die Exuvien europäischer Libellen: The Exuviae of European dragonflies*. Jena, Arnika & Eisvogel, Höxter.
- Ivanov, V.P. (2000). *Sensory organs of insects and other arthropods*. Moscow, Nauka. [In Russian].
- Klass, K.-D. (2008). The female abdomen of ovipositor-bearing Odonata (Insecta: Pterygota). *Arthropod Systematics & Phylogeny*, 66, 45–142.
- Linksvayer, T.A. & Wade, M.J. (2005). The evolutionary origin and elaboration of sociality in the aculeate Hymenoptera: maternal effects, sib-social effects, and heterochrony. *The Quarterly Review of Biology*, 80, 515–516.
- Machado, A.B.M. & Costa, J.M. (1995). *Navicordulia* gen. nov. a new genus of Neotropical Corduliidae, with descriptions of seven new species (Anisoptera: Corduliidae). *Odonatologica*, 24, 187–218.
- Machotin, A. (1929). Zur morphologischen Bedeutung der äußeren Geschlechtanhänge von Odonata und *Carausius morosus* Br. *Zoologicheskii Jurnal*, 9, 23–84. [In Russian; Germany summary].
- Machotin, A. (1953). Phylogenetic relationships of principal groups of saltatorian and morphology of their ovipositor. *Proceedings of the Institute of Animal Morphology ANSSSR*, 8, 5–62. [In Russian].
- Matsuda, R. (1987). *Animal evolution in changing environments, with special reference to abnormal metamorphosis*. New York: Wiley.
- Matushkina, N.A. (2004). [Comparative morphology of ovipositor in some damselflies (Odonata, Zygoptera). In Russian; English summary and captions]. *Vestnik zoologii*, 38(3), 53–66.
- Matushkina, N.A. (2008a). Skeleto-muscular development of genital segments in the dragonfly *Anax imperator* (Odonata, Aeshnidae) during metamorphosis and its implications for the evolutionary morphology of the insect ovipositor. *Arthropod Structure and Development*, 37, 321–332.
- Matushkina, N.A. (2008b). The ovipositor of the relic dragonfly *Epiophlebia superstes*: a morphological re-examination (Odonata: Epiophlebiidae). *International Journal of Odonatology*, 11, 71–80.
- Matushkina, N.A. & Gorb, S.N. (1997). Skeleton-muscle organisation of the endophytic ovipositor in Odonata. *Vestnik zoologii*, 31(5–6), 57–70. [In Russian; English summary and captions].
- Matushkina, N.[A.] & Gorb, S.[N.] (2002). Stylus of the odonate endophytic ovipositor: a mechanosensory organ controlling egg positioning. *Journal of Insect Physiology*, 48, 213–219.
- Matushkina, N.[A.] & Gorb, S.[N.] (2007). Mechanical properties of the endophytic ovipositor in damselflies (Zygoptera, Odonata) and their oviposition substrates. *Zoology*, 110, 167–175.
- Matushkina, N.A. & Klass, K.-D. 2011. Morphology of female external genitalia in *Phenes raptor* (Odonata: Petaluridae). *International Journal of Odonatology*, 14(3), 199–215.
- Matushkina, N.A. & Lambret, P.H. (2011). Ovipositor morphology and egg laying behaviour in the dragonfly *Lestes macrostigma* (Zygoptera: Lestidae). *International Journal of Odonatology*, 14, 69–82.
- Miller, P.L. (1989). Possible functions of the sub-genital plates of female libellulid dragonflies (Anisoptera: Libellulidae). *Advances in Odonatology*, 4, 57–71.
- Nalepa, C.A. & Bandi, C. (2000). Characterizing the ancestors: paedomorphosis and termite evolution. In T. Abe, D.E. Bignell, and M. Higashi (Eds.) *Termites: Evolution, Sociality, Symbioses, Ecology* (pp. 53–75). Boston: Kluwer Academic Publishers.
- Paulson, D.R. (1969). Oviposition in the tropical dragonfly *Micrathyria Tombo*, 12, 12–16.

- Pfau, H.K. (1985). Die eigentümliche Eiablage der *Cordulegaster*-Weibchen. *Natur und Museum*, 5, 77–86.
- Pfau, H.K. (1991). Contributions on functional morphology to the phylogenetic systematics of Odonata. *Advances in Odonatology*, 5, 109–141.
- Radford, A.P. (1995). *Sympetrum sanguineum* (Müller) ovipositing on dry land. *Journal of the British Dragonfly Society*, 11(2): 30.
- Rowe, R.J. (1988). Alternative oviposition behaviours in three New Zealand corduliid dragonflies: their adaptive significance and implications for male mating tactics. *Zoological Journal of the Linnean Society, London*, 92, 43–66.
- Schiemenz, H. (1953). *Die Libellen unserer Heimat*. Jena: Urania.
- Schorr, M. (1990). *Grundlagen zu einem Artenhilfsprogramm Libellen der Bundesrepublik Deutschland*. Bithoven: Ursus.
- Schorr, M., Schneider, W. & Dumont, H.J. (1998). Ecology and distribution of *Lindenia tetraphylla* (Insecta, Odonata, Gomphidae): a review. *International Journal of Odonatology*, 1, 65–88.
- St. Quentin, D. (1962). Der Eilegeapparat der Odonaten. *Zeitschrift für Morphologie und Ökologie der Tiere*, 51, 165–189.
- Suhling, F. & Müller, O. (1996). *Die Flußjungfern Europas. Gomphidae. Die Neue Brehm-Bücherei* 628. Magdeburg: Westarp & Spektrum.
- Tillyard, R.J. (1917). *The Biology of Dragonflies*. Cambridge: Cambridge University Press.
- van der Weele, H.W. (1906). Morphologie und Entwicklung der Gonapophysen der Odonaten. *TijdschRift voor Entomologie*, 49, 99–198.
- Vinicius, L. & Lahr, M.M. (2003). Morphometric heterochrony and the evolution of growth. *Evolution*, 57, 2459–2468.
- Wildermuth, H. (1994). Habitatselektion bei Libellen. *Advances in Odonatology*, 6, 223–257.
- Zacharuk, R.Y. (1980). Ultrastructure and function of insect chemosensilla. *Annual Review of Entomology*, 25, 27–47.
- Zeh, D.W., Zeh, J.A. & Smith, R.L. (1989). Ovipositors, amnions and eggshell architecture in the diversification of terrestrial arthropods. *Quarterly Review of Biology*, 64, 147–168.